



Electron Transfer between the Gram-positive *Enterococcus faecalis* Bacterium and Electrode Surface through Osmium Redox Polymers

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Electron Transfer between the Gram-positive *Enterococcus faecalis* Bacterium and Electrode Surface via Osmium Redox Polymers

Galina Pankratova,^{*,[a]} Ewelina Szypulska,^[b] Dmitry Pankratov,^[c] Dónal Leech,^[d] and Lo Gorton^{*,[a]}

Abstract: The interaction between microorganisms, that contain thick cell walls such as the Gram-positive bacterium *Enterococcus faecalis*, and conductive surfaces can be improved using redox polymers as mediators. Here we report on a detailed electrochemical characterization of the communication between *E. faecalis* cells and a series of osmium redox polymers with different redox potentials. The current generated from glucose oxidation by films containing osmium redox polymers and *E. faecalis* cells increase over time (28 h) due to a decrease in charge transfer resistance within the films, possibly due to improved penetration of the redox polymer within the bacterial cell wall.

Over the past several decades microbial electrochemistry has emerged as a hot research topic in environmental engineering technologies, microbial ecology and microbial physiology.^[1] The considerable attention to various electrochemically active microorganisms has been due to potential biotechnological applications, both current producing and current consuming, arising from the possibility of connecting cell metabolism to an electrode. Research on microbial electron exchange reactions associated with solid materials can also provide for a better understanding of biogeochemical cycles and cell-to-cell electrical communication.^[2,3]

Microbe-electrode interconnection is driven by a microbial metabolic process, known as extracellular electron transfer (EET). Achieving efficient electrochemical interaction between a certain microbial cell and a conductive surface, quantitatively described as the ET rate, is a challenging complex task dependent on a number of factors.^[3] There are a few known approaches to enhance EET rates at the cell-electrode interface through promoting direct or mediated ET.^[4] Direct ET ensures a minimized overpotential and coulombic losses in combination with a simplified operational design. Gram-positive bacteria and yeasts have a thick cell wall that impair the capacity for effective direct ET; to improve the interaction with the extracellular environment for those microorganisms utilization of artificial mediating agents is required. Use of osmium (Os) redox

polymers (RPs) has a number of advantages over use of soluble freely diffusing mediators. These include formation of a high local concentration of a redox network, a stable multilayered 3D-hydrogel serving as a matrix for further “wiring” biocatalysts, and simplified design of constructed systems in contrast to use of diffusing mediators.^[5] Originally Os-based electron-conducting polymers were demonstrated as mediators facilitating ET between redox enzymes and electrodes.^[6] The very first “wired” using an Os RP matrix was bacterium *Gluconobacter oxydans* as reported in 2004.^[7] Since that time several bacteria, both Gram-negative^[8] and Gram-positive,^[9] have been electrochemically connected to various electrode materials via Os-based mediators. Among others, the Gram positive bacterium *Enterococcus faecalis* has recently been demonstrated to perform EET to gold electrodes mediated by a Os-based polymeric shuttle.^[9b] In the current work we continue our investigation on the electrochemical communication between *E. faecalis* cells and Os-based polymeric mediators and report, to the best of our knowledge, the very first detailed characterization of ET in the system Gram-positive bacterium-Os RP-electrode.

Herein we have tested three different Os RP as mediators: [Os(4,4'-dimethyl-2,2'-bipyridine)₂(poly-vinylimidazole)₁₀Cl]⁺²⁺ (Os-1),^[10] [Os(2,2'-bipyridine)₂(poly-vinylimidazole)₁₀Cl]⁺²⁺ (Os-2),^[11] [Os(4,4'-dicholo-2,2'-bipyridine)₂(poly-vinylimidazole)₁₀Cl]⁺²⁺ (Os-3).^[12] The Os RPs have analogous chemical structures, but with various ligands coordinating the redox center of the polymers and thus varying redox potentials, E^0 , in the range from 0.320 to 0.490 V (Table 1). The E^0 values were estimated by taking the mean value of the anodic and cathodic peak potentials of the recorded cyclic voltammograms (CVs) (Figure S1). Note, that all potential values in this work are reported versus the standard hydrogen electrode (SHE).

To prepare Os RP modified electrodes a 5 μ L aliquot of an aqueous polymer solution (10 mg mL⁻¹) was dropcast on a polished graphite rod (Alfa Aesar GmbH, Germany, \varnothing 3.05 mm) surface and dried at room temperature for 30 min. In the case of microbial modification of the electrodes an additional layer of 1 μ L of an *E. faecalis* cell suspension (1 g mL⁻¹, wet weight) was applied on the top of the Os RP modified electrodes and dried again for 10 min. In order to prevent the leakage of the applied material all electrodes were covered with a dialysis membrane presoaked in buffer (Spectrum Laboratories Inc., USA, 6000-8000 Da cutoff). All experiments were performed in a 50 mM phosphate buffer containing 0.1 M KCl (pH 7.40). For a detailed description of the experimental procedures, see the Supporting Information (SI).

The electrochemical behavior of the Os RP modified electrodes was characterized by CV. The CVs recorded at different scan rates (v , 0.0005 to 1.0 V s⁻¹) display a well-defined pair of oxidation and reduction peaks (Figure S1). The quantity of redox active Os on the electrode surface was calculated from CVs by integration of the area under the redox peak; the surface

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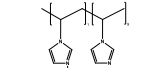
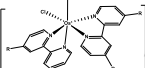

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coverage, Γ , values are presented in Table 1. Since diffusion-controlled processes are significant in ET of the Os RPs,^[13] the Nicholson method^[14] was applied to estimate apparent ET rate constants, k_{app} (Figure S2), cognisant that uncompensated resistance can influence this estimate. More details can be found in the SI. The estimated k_{app} value (Table 1) is highest for the Os-1 modified electrodes even though the Os-1 polymer has the lowest E^0 in the selection, 0.33 V. Os-1 polymer was not however the best in accepting electrons from the *E. faecalis* cells among the others. Background subtracted anodic waves of CVs for the bacterial cells mediated by the various Os RPs in response to D-glucose are shown in Figure 1a. They were obtained as the difference between the anodic waves recorded in 10 mM D-glucose and buffer solution. Control CV experiments for the Os RP modified electrodes in the absence/presence of D-glucose were performed (Figure 1a, dashed curves). Very little current response (about 1–2 $\mu\text{A cm}^{-2}$) was observed from the Os RPs, which indicates the almost total absence of parasitic current input from the used Os mediators. When the cells were applied, well-pronounced bioelectrocatalytic currents occurred for all the Os RP modified electrodes at a potential higher than 0.2 V (Figure 1a). The highest current generation ($51 \pm 2 \mu\text{A cm}^{-2}$ at 0.6 V) was observed with Os-2, which had the marginally lowest estimated ET rate constant with the electrode. This polymer also displayed the best results in terms of generated current output for all the biocatalysts tested using the same mediator selection, viz. algae,^[15] cyanobacteria,^[16] purple nonsulfur bacterium,^[17] thylakoid membranes,^[18] and glucose dehydrogenase.^[19] The lowest current density was registered for the Os-3 polymer even though it has the highest E^0 value. Such an affect could be attributed to a decreased Γ of the electrode by the polymer (Table 1) and thus, less active electrochemical mediator capacity.

Table 1. Electrochemical characteristics of the studied Os RP (E^0 , Γ , k_{app}) and *E. faecalis* cells–Os RP interaction (R_{ct}).

Os RP	Structure	R	E^0 / V	Γ / 10^{-6} / mol cm^{-2}	k_{app} / $\text{s}^{-1/2}$	R_{ct} / k Ω
Os-1		CH ₃	0.327 ± 0.004	0.97 ± 0.13	3.44 ± 0.45	6.93 ± 0.91
			0.424 ± 0.003	1.95 ± 0.16	2.26 ± 0.19	5.53 ± 0.51
Os-2		H	0.490 ± 0.010	0.92 ± 0.05	2.44 ± 0.09	14.43 ± 2.02
			0.490 ± 0.010	0.92 ± 0.05	2.44 ± 0.09	14.43 ± 2.02
Os-3		Cl	0.490 ± 0.010	0.92 ± 0.05	2.44 ± 0.09	14.43 ± 2.02

Time [a] and [b] corresponds to experimental data obtained immediately after fabrication of the electrodes and after the long-term amperometric treatment (28 h), respectively.

To study the ET processes occurring in the *E. faecalis* cells–Os RP system, electrochemical impedance spectroscopy (EIS) experiments were carried out at 0.6 V in the presence of 10 mM

D-glucose. The applied potential value was chosen to ensure a potential-independent response for all the patterns. Furthermore, maximum current generation by the cells was observed for all the Os RP modified electrodes at this particular potential (Figure 1a). The Nyquist plots of the experimental data were fitted by employing an equivalent circuit model presented in Figure 2a (inset), which has successfully been applied for the characterization of conducting polymer-modified electrodes^[20] as well as for the electrochemical behavior of Gram-positive bacteria.^[21] In the proposed circuit, R_s is the ohmic solution resistance, R_p represents the polarization resistance, the charge transfer resistance is given by R_{ct} , the effect from the non-ideal double layer electrode capacitance and pseudocapacitance is represented by the two constant phase elements, CPE_{dl} and CPE_{ϕ} , respectively. The accuracy of the fitting in respect to the experimental data is confirmed by a satisfactory approximation, χ^2 , which was about 10^{-4} for all data fittings (Table S1). The monitored R_{ct} values, indirect evaluation of the ET reaction rate, are listed in Table 1. As can be seen from the EIS data fitting,

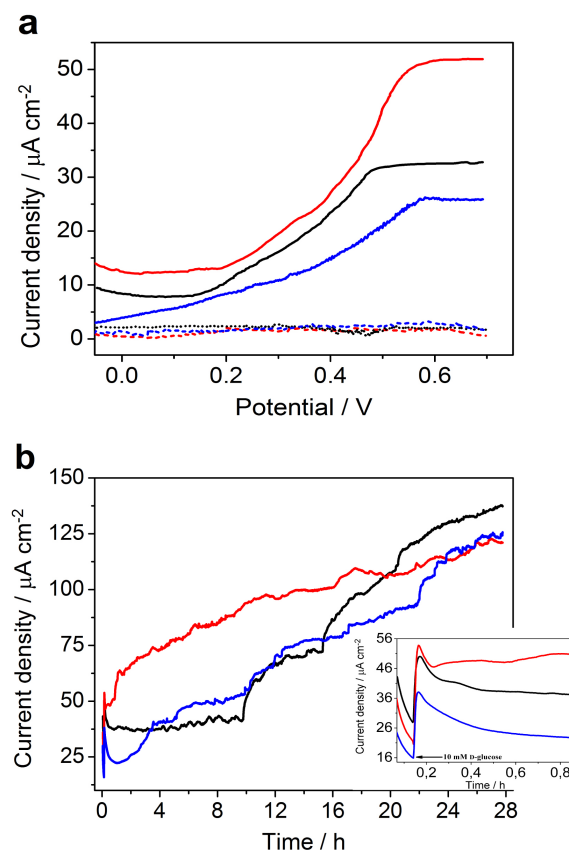


Figure 1. Electrochemical communication between *E. faecalis* cells and graphite electrode surface mediated by Os-1 (black), Os-2 (red) and Os-3 (blue) in the presence of 10 mM D-glucose: (a) Background subtracted anodic cyclic voltammetric waves obtained as a difference between anodic waves recorded in buffer with and without 10 mM D-glucose (solid curves). Dashed curves show catalytic current generated by Os RPs in the presence of 10 mM D-glucose. Scan rate 0.5 mV s^{-1} ; (b) Long-term amperometry measurements at constantly applied potential of 0.6 V. The inset shows the initial period of the amperometric measurements when D-glucose has just been added. All potentials are given vs. SHE.

the best ET was achieved for Os-2 with the lowest R_{ct} of $5.53 \pm 0.51 \text{ k}\Omega$, which may explain the highest current response from the *E. faecalis* cells when employing it as a mediator compared to the other Os RP. The obtained R_{ct} magnitude is in complete agreement with the current-generating trend (Figure 1a) of bacterial cells mediated by different Os polymers. However, R_{ct} values for the system bacterial cell–Os polymer–electrode do not correlate with the k_{app} values within the polymeric matrix. This suggests that the electrochemical communication between the microbial cells and the Os RPs is limited by the ET rate between the *E. faecalis* cells and the Os-based mediators, but not by the ET at the electrode–Os RP interface.

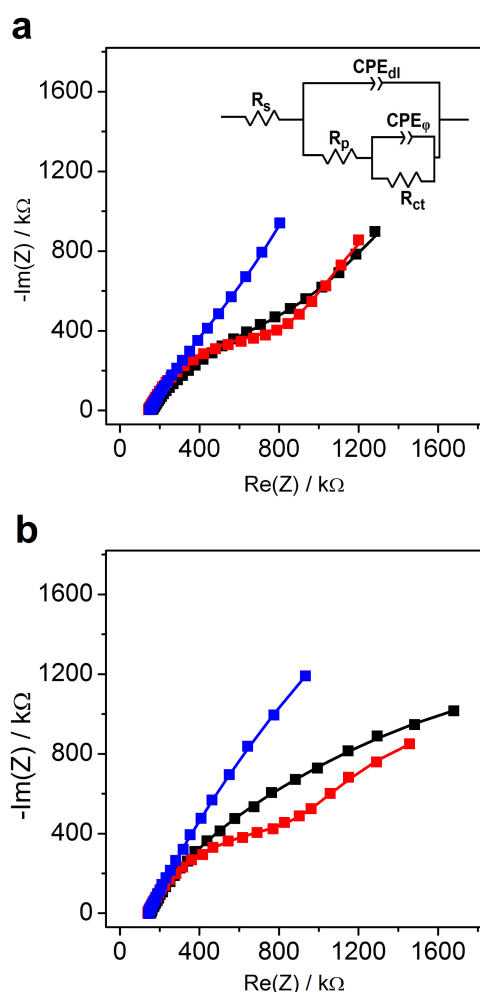


Figure 2. Representative Nyquist plots of *E. faecalis*–Os RP (Os-1 (black), Os-2 (red) and Os-3 (blue)) interaction obtained at 0.6 V vs. SHE in the presence of 10 mM D-glucose (a) immediately after fabrication of the electrodes and (b) after the long-term amperometric measurements. The solid lines represent the equivalent circuit fitting, whereas the points represent the experimental data. The inset shows the equivalent circuit model used in the fitting, representing electrolyte resistance (R_s), charge transfer resistance (R_{ct}), polarization resistance (R_p), pseudocapacitance and non-ideal double layer, introduced by two constant phase elements (CPE_{dl} and CPE_{ϕ} , respectively).

Long-term amperometric measurements of the bacterial cells mediated by the various Os RPs were carried out at a constant potential of 0.6 V in the presence of 10 mM D-glucose (Figure 2b). In response to the provided electron donor the immobilized *E. faecalis* cells initiate biocatalytic current generation of various intensity for the used mediators (Figure 2b, inset). The general trend of the current-producing dependence on the structure of the employed Os RP was in good agreement with the obtained CV and EIS results, as well as with the previously reported amperometric investigations for biocatalysts of different nature.^[16–18] During the first hours of the experiments a decrease in current response to different extent for the Os RPs is observed (Figure 2b). This might be related to the bacteria's adaptation to the new environment. Nevertheless, within the next few hours, a clear continuous current gain with intensity in accordance with the order of the reported R_{ct} values for the studied variations was observed. Bacterial cells mediated by Os-2 produced the highest current density. However, after about 19 to 21 h the order is inverted to conform with the k_{app} reported trend for the Os RP. To gain insight into the complex situation, additional electrochemical characterization of the modified electrodes was performed by EIS measurements under the same condition as before immediately after the long-term amperometry tests (Figure 2b). After 28 h of amperometry, the R_{ct} values significantly decreased compared to the initial measurements, which explains the enhanced electron transfer and consequently the rising current generation from the bacterial cells over time for all the Os RP. It cannot be attributed to the bacterial growth on the electrode, since the microbes were supplied only with glucose. All enterococci are nutritionally fastidious and require a complex medium, containing amino acids and vitamins.^[22] The fact that the values scale with k_{app} for Os RPs could indicate that the efficiency of ET from *E. faecalis* cells to graphite is now limited by the ET rate of conducting electrons from the Os RP to the electrode, but not by the microbe–mediator interaction. The change in the ET limiting processes might derive from the Os RP ability to penetrate into the bacterial cell over time. The difference in ET efficiency for the Os RPs might also be related to a different penetration ability of the redox polymeric mediators due to their structure.

In conclusion, mediated microbe–electrode interaction is a complex phenomenon. For this reason complementary interdisciplinary research is required to understand the main features of microbial EET nature. Results presented in this study provide an electrochemical description of the *E. faecalis* cells–Os RP–electrode surface system. The initial electrochemical communication is limited by the interaction between the cells and the Os-based mediators. With time the system performance overcomes the limitation and becomes defined by the ET rates between the Os polymers and the electrode. Taking into consideration all the data, we assume that the penetration of the Os RPs into the bacterial cell wall over time is one of the possible explanations. Electroactive Gram-positive bacteria are still a “black box” when it comes to the electrode associated biochemical mechanisms. However, we believe that our current findings may contribute to a mechanistic understanding of the EET processes in the future. Further research focusing on the aspects of the EET between Gram-positive *E. faecalis* bacteria and conductive surfaces is now in progress in the laboratory.

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Keywords: Gram-positive bacterium • *Enterococcus faecalis* • osmium redox polymers • electrochemical communication • extracellular electron transfer

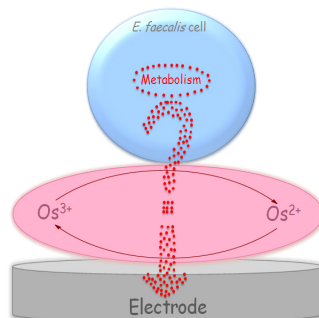
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COMMUNICATION

**Along the microbe-electrode
electron exchange pathway:**

Electron transfer features of the
Gram-positive bacterium

Enterococcus faecalis via osmium
redox polymers of various structure
and redox potentials are characterized
in details using electrochemical
techniques.



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Page No. 1 – Page No. 4

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